



NTP
National Toxicology Program

Toxicology and Carcinogenesis Studies of Pulegone in F344/N Rats and B6C3F1 Mice (Gavage Study)

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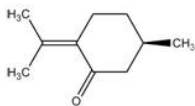
National Institute of Environmental Health Sciences

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Pulegone

- Nominated by NIEHS based on the potential for human exposure and the absence of carcinogenicity data
- Found in several essential oils that are used to provide **mint** flavoring for foods, drinks, and dental products. Also found in select herbal medicines
- Some synthetic production, however most exposure comes through its natural occurrence in food
- Use of certain herbal medicines can lead to exposures as high as 2.3 mg/kg/day





Experimental Design

Genotoxicity:	Ames mutagenicity and Erythrocyte micronucleus (mice)
ADME Study:	0.8, 8.0 and 80 mg/kg in male and female B6C3F1 mice and F344/N rats
14-day Study:	Rats: 0, 37.5, 75, 150, 300, 600 mg/kg Mice: 0, 18.75, 37.5, 75, 150, 300 mg/kg (5 animals/species/sex/dose)
13-week Study:	Rats and mice: 0, 9.375, 18.75, 37.5, 75, 150 mg/kg (10 animals/species/sex/dose)
2-year Study:	Male rats: 0, 18.75, 37.5, 75 mg/kg Female rats, male and female mice: 0, 37.5, 75, 150 mg/kg (50 animals/species/sex/dose)



NTP Genotoxicity Test Results

- Ames mutagenicity test – negative (with and without S9)
- Micronucleus test, male and female mice – negative (3-month study)



ADME TK Studies

- Rapidly and extensively absorbed from the gastrointestinal tract
- Male rats tend to have higher tissue concentrations compared to female rats, especially in kidney
 - Pulegone binds reversibly to α_2 -globulin
 - No accumulation of α_2 -globulin observed in kidney
 - Sex difference is not seen in mice
- Metabolic profile is complex: at least three pathways involving hydroxylation, reduction, or conjugation with glutathione as first steps.
- Primarily excreted in the urine
- Mice exhibit slightly higher rates of clearance
- $T_{1/2}$ is approximately 2 hours



14-Day Study in Rats

	Vehicle control	37.5	75	(mg/kg) 150	300	600
<i>Males</i>						
Survival	5	5	5	5	0	0
Final body weight (% control)		90**	97	88**	-	-
Liver necrosis	0	-	-	1(1.0) ^a	5**(2.2)	4*(1.8)
<i>Females</i>						
Survival	5	5	5	5	1	0
Final body weight (% control)		107	100	103	71	-
Liver necrosis	0	-	0	0	4**(2.0)	5**(1.4)

N=5

*P<0.05, **P<0.01

^aSeverity



Dose Selection Rationale for 13-Week Study - Rats

- Doses by gavage set at: 9.375, 18.75, 37.5, 75, and 150 mg/kg
- Based on mortality and liver necrosis observed in the 14-day study at doses of 300 and 600 mg/kg



13-Week Study in Rats

	Vehicle control	9.375	18.75	(mg/kg) 37.5	75	150
<i>Males</i>						
Survival	10	10	10	10	10	10
Final body weight (% control)		100	97	99	91**	69**
<i>Females</i>						
Survival	10	10	10	10	10	9
Final body weight (% control)		101	97	96	97	89**

N= 10

**P<0.01



13-Week Study in Rats – Select Histopathology

	Vehicle control	9.375	18.75	(mg/kg) 37.5	75	150
<i>Males</i>						
Hyaline Glomerulopathy (Kidney)	0	0	0	0	2(1.0)	10** (1.0)
Hepatocyte Focal Necrosis (Liver)	0	0	0	0	0	6** (1.0)
Bile Duct Hyperplasia (Liver)	0	0	0	0	9** (1.0)	10** (2.0)
Periportal Fibrosis (Liver)	0	0	0	0	0	10** (1.0)
<i>Females</i>						
Hyaline Glomerulopathy (Kidney)	0	0	0	0	0	8** (1.0)
Bile Duct Hyperplasia (Liver)	0	0	0	0	1(1.0)	10** (1.7)
Periportal Fibrosis (Liver)	0	0	0	0	0	9** (1.0)

N=10

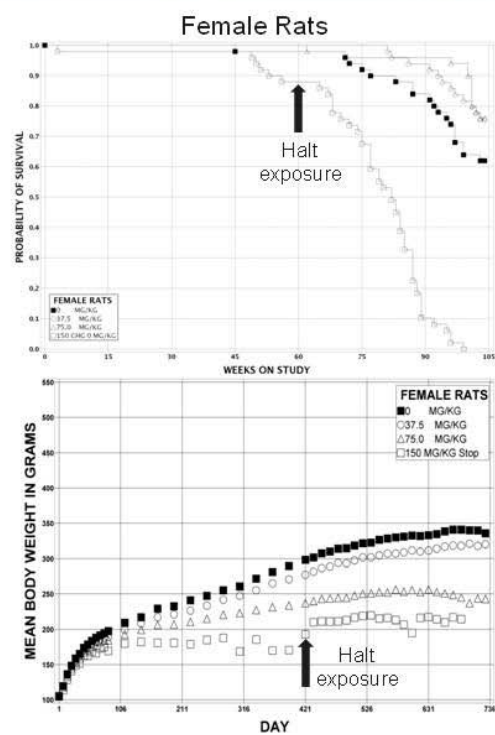
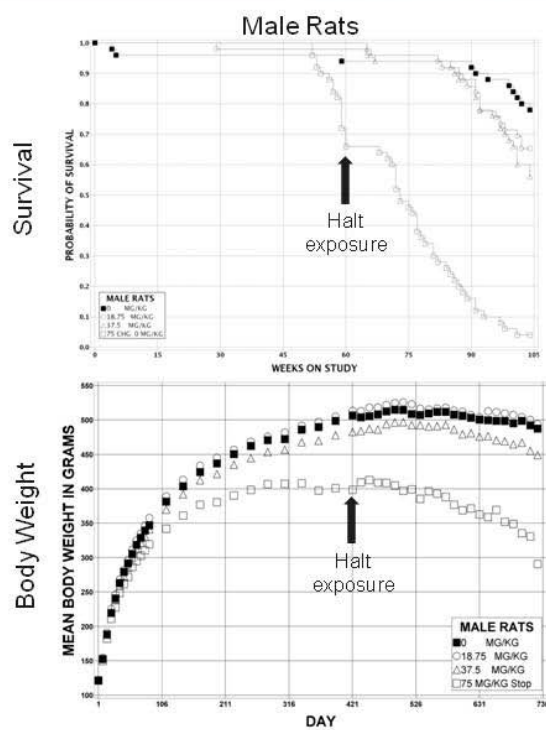
** P<0.01

* Severity



Dose Selection Rationale for 2-Year Study - Rats

- Highest dose levels for the 2-year studies were 75 mg/kg for male rats and 150 mg/kg for female rats
- Based on:
 - In the 13-week study the final body weight of the 150 mg/kg males was 30% less than that of controls and hepatic necrosis was observed
 - The final body weight of the 75 mg/kg males and 150 mg/kg females from the 13 week study was about 10% less than their respective vehicle controls
 - Considered adequate high doses





Cause of Increased Mortality

- Renal failure secondary to hyaline glomerulopathy and chronic progressive nephropathy (CPN)

	Vehicle control	18.75	(mg/kg) 37.5	75	150
<i>Males</i>					
Hyaline Glomerulopathy	0/50	0/50	9/50 ^{**} (1.1)	24/50 ^{**} (1.6)	-
CPN	45(1.9)	45(1.9)	50(2.9)	50(4.0)	-
<i>Females</i>					
Hyaline Glomerulopathy	0/50	-	17/50 ^{**} (1.0)	49/50 ^{**} (2.2)	48/49 ^{**} (3.3)
CPN	42(1.2)	-	44(1.3)	49 ^{**} (2.9)	48 ^{**} (3.4)

^{**} P<0.01

^a Severity



Neoplastic Effects - Rat

- Male rat: none
- Female rat: urinary bladder

	Vehicle control	37.5	(mg/kg) 75	150
Urinary Bladder Papilloma	0/50	0/49	1/50	3/47 [*]
Urinary Bladder Papilloma or Carcinoma ^a	0/50	0/49	1/50	5/47 ^{a,b}

^a Historical incidence for 2-year gavage studies with corn oil vehicle control groups: 0/200; all routes: 0/1,347

^b Survival adjusted rate: 20.8%

^{*} P<0.05



Non-Neoplastic Effects - Rat

- Male and female
 - Liver (Necrosis and Portal fibrosis among many other lesions)
 - Kidney (Hyaline glomerulopathy among other lesions)
 - Nose (Olfactory epithelium degeneration among other lesions)
- Male only
 - Forestomach (Ulceration among other lesions)

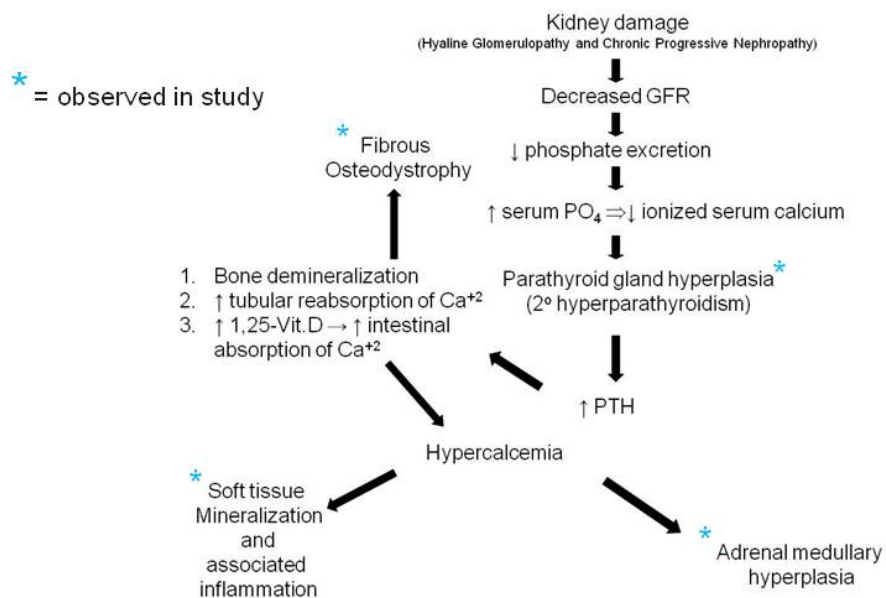


Pathology Considered Secondary to Renal Disease

- Parathyroid gland hyperplasia
- Fibrous osteodystrophy
- Blood vessel and tissue calcification and associated inflammation
- Adrenal medullary hyperplasia



Hypothetical Relationship Between Secondary Lesions





14-Day Study in Mice

	Vehicle control	18.75	37.5	(mg/kg) 75	150	300
<i>Males</i>						
Survival	5	5	5	5	5	4
Final body weight (% control)		105	102	100	103	98
Liver necrosis	1(1.0)	0	0	0	1(2.0)	5*(2.0)
<i>Females</i>						
Survival	5	5	5	5	5	1
Final body weight (% control)		106	104	102	101	96
Liver necrosis	1(1.0)	0	0	0	0	4*(3.0)

N=5

*P<0.05



Dose Selection Rationale for 13-Week Study - Mice

- Doses selected for the 13-week gavage study in mice were 9.375, 18.75, 37.5, 75, and 150 mg/kg
- Based on mortality and liver necrosis at 300 mg/kg in the 2-week study



13-Week Study in Mice

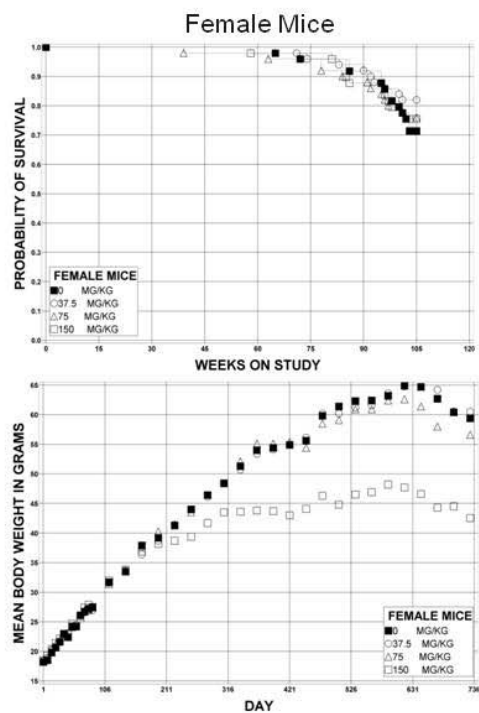
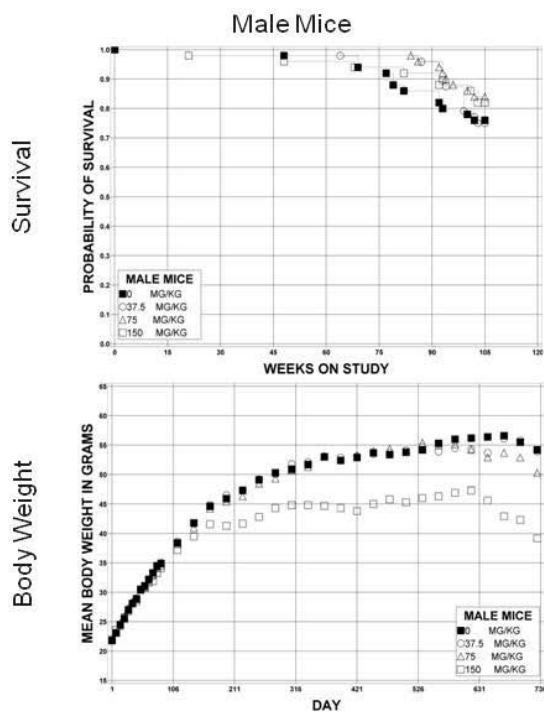
	Vehicle control	9.375	18.7	(mg/kg) 37.5	75	150
<i>Males</i>						
Survival	10	10	10	10	10	10
Final body weight (% control)		99	102	98	98	99
<i>Females</i>						
Survival	10	10	10	10	10	10
Final body weight (% control)		102	101	109	103	96
N=10						

*No chemical-related histological changes were observed



Dose Selection Rationale for 2-year Study - Mice

- Doses selected for the 2-year gavage study were 37.5, 75, and 150 mg/kg
- Based on the lack of mortality and effects on body weights and the lack of lesions attributable to pulegone administration in the 13 week study





Neoplastic Effects - Male Mice

	Vehicle control	37.5	(mg/kg) 75	150
Hepatocellular adenoma, multiple	6/50	19/50**	27/50**	18/50**
Hepatocellular adenoma (includes multiple) ^a	22/50	31/50	35/50**†	28/50
Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma ^b	29/50*	37/50	42/50**†	36/50

^a Historical control range for 2-year gavage studies with corn oil: 44%-54%; all routes: 24%-72%

^b Historical control range for corn oil gavage studies: 58%-76%; all routes: 46%-92%

* P<0.05

** P<0.01

† Exceeds historical control range for corn oil gavage



Neoplastic Effects - Female Mice

	Vehicle control	37.5	(mg/kg) 75	150
Hepatocellular adenoma ^a	13/49**	15/50	13/50	27/50** [†]
Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma ^b	17/49**	15/50	15/50	33/50** ^{†#}
Osteoma or osteosarcoma, all organs ^c	0/49	0/50	3/50 [†]	1/50

^a Historical control range for corn oil gavage studies: 6%-27%; all routes: 2%-62%

^b Historical control range for corn oil gavage studies: 8%-35%; all routes: 6%-64%

^c Historical control range for corn oil gavage studies: 0%-2%; all routes: 0%-4%

** P<0.01

[†] Exceeds historical control range for corn oil gavage

[#] Exceeds historical control range for all routes



Non-Neoplastic Effects - Mice

- Male and female
 - Liver (Necrosis and eosinophilic foci among many other lesions)
 - Kidney (Hyaline glomerulopathy among other lesions)
 - Nose (Olfactory epithelium degeneration among other lesions)
 - Forestomach (Hyperplasia and inflammation)



Conclusions

- Male rats: no evidence of carcinogenic activity
- Female rats: some evidence of carcinogenic activity – urinary bladder
- Male mice: clear evidence of carcinogenic activity - liver
- Female mice: clear evidence of carcinogenic activity – liver
equivocal evidence of carcinogenic activity – bone
- Increased non-neoplastic lesions:
 - Male and female rats and mice
 - Kidney (hyaline glomerulopathy), liver and nose
 - Male rats and mice and female mice
 - Forestomach



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Questions?

